

Arabidopsis Extra Large G-Protein 2 (XLG2) Interacts with the G β Subunit of Heterotrimeric G Protein and Functions in Disease Resistance

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ABSTRACT Heterotrimeric GTP-binding proteins, which consist of G α , G β , and G γ subunits, play important roles in transducing extracellular signals perceived by cell surface receptors into intracellular physiological responses. In addition to a single prototypical G α protein (GPA1), *Arabidopsis* has three unique G α -like proteins, known as XLG1, XLG2, and XLG3, that have been found to be localized in nuclei, although their functions and mode of action remain largely unknown. Through a transcriptomic analysis, we found that XLG2 and XLG3 were rapidly induced by infection with the bacterial pathogen *Pseudomonas syringae*, whereas the XLG1 transcript level was not affected by pathogen infection. A reverse genetic screen revealed that the *xlg2* loss-of-function mutation causes enhanced susceptibility to *P. syringae*. Transcriptome profiling revealed that the *xlg2* mutation affects pathogen-triggered induction of a small set of defense-related genes. However, *xlg1* and *xlg3* mutants showed no difference from wild-type plants in resistance to *P. syringae*. In addition, the *xlg2 xlg3* double mutant and the *xlg1 xlg2 xlg3* triple mutant were not significantly different from the *xlg2* single mutant in the disease resistance phenotype, suggesting that the roles of XLG1 and XLG3 in defense, if any, are less significant than for XLG2. Constitutive overexpression of XLG2 leads to the accumulation of abnormal transcripts from multiple defense-related genes. Through co-immunoprecipitation assays, XLG2 was found to interact with AGB1, the sole G β subunit in *Arabidopsis*, which has previously been found to be a positive regulator in resistance to necrotrophic fungal pathogens. However, no significant difference was found between three *xlg* single mutants, the *xlg2 xlg3* double mutant, the *xlg* triple mutant, and wild-type plants in resistance to the necrotrophic fungal pathogens *Botrytis cinerea* or *Alternaria brassicicola*. These results suggest that XLG2 and AGB1 are components of a G-protein complex different from the prototypical heterotrimeric G-protein and may have distinct functions in modulating defense responses.

Key words: Defense responses; disease resistance; plant–microbe interactions; *Arabidopsis*; G-protein.

INTRODUCTION

Heterotrimeric guanine nucleotide-binding proteins (G-proteins) transduce a wide array of extracellular signals into appropriate physiological responses (Neer, 1995; Neves et al., 2002). The signaling pathway is initiated by binding of an extracellular ligand to a cell surface receptor that belongs to the superfamily of seven transmembrane G-protein-coupled receptors (GPCRs). The recognition of the ligand by the receptor leads to activation of the G-protein, which is made up of three subunits: G α , G β , and G γ . G α and G $\beta\gamma$ subunits then dissociate and activate their respective downstream effectors, which include phospholipases, nitric oxide synthase, adenylyl cyclase, ion channels, MAPKs, Ca²⁺ channels, and other proteins

(Neer, 1995; Neves et al., 2002; Cabrera-Vera et al., 2003). Although mammals have nearly 1000 GPCRs and 20 G α , 5 G β , and 12 G γ isoforms, plants have a much smaller number of G-protein components (Assmann, 2004; Temple and Jones, 2007). For instance, the *Arabidopsis* genome only

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encodes one canonical $G\alpha$ (GPA1), one $G\beta$ (AGB1), and two known $G\gamma$ subunits (AGG1 and AGG2) (Ma et al., 1990; Weiss et al., 1994; Mason and Botella, 2000, 2001), and the number of predicted GPCRs is also much smaller (Moriyama et al., 2006; Gookin et al., 2008). Despite the paucity of G-protein components, mutational analyses have revealed that G-protein signaling functions in a variety of biological processes in plants, including the auxin response, ABA-mediated inhibition of stomatal opening, cell division and expansion, selected light responses, seed germination, sugar sensing, and drought tolerance (reviewed by Jones and Assmann, 2004; Perfus-Barbeoch et al., 2004). Several studies have also indicated that different G-protein subunits play distinct roles in disease resistance (Suharsono et al., 2002; Llorente et al., 2005; Trusov et al., 2006, 2007; Zhang et al., 2008).

Unlike animals, plants do not have specified cells to defend themselves against pathogen attack. Instead, every living plant cell is generally equipped with the components necessary for detecting invading pathogens and mounting an appropriate defense response. A plant cell contains receptors that recognize conserved microbe-/pathogen-associated molecular patterns (MAMPs/PAMPs) (Gomez-Gomez and Boller, 2000; Nurnberger et al., 2004; Ausubel, 2005; Kaku et al., 2006; Zipfel et al., 2006; Wan et al., 2008). The MAMP-triggered innate immune response provides the first layer of induced defense against an invading pathogen. This non-race-specific basal resistance, together with constitutive physical and chemical barriers, successfully prevents most infections from becoming established. To overcome basal resistance, pathogens have evolved a repertoire of virulence effector proteins that are delivered into hosts to suppress the basal defense response (Abramovitch and Martin, 2004; da Cunha et al., 2007). In turn, plants have evolved Resistance (R) proteins, each of which recognizes the action of specific virulent effector(s) as a signal of invasion to trigger the hypersensitive response (HR) (Jones and Dangl, 2006). HR is a strong physiological response that often leads to cell suicide and elimination of the pathogen.

Growing evidence indicates that the basal defense response largely overlaps with the R-protein-mediated HR, and that R-proteins may function to hyper-activate the basal resistance mechanism (Tao et al., 2003; Navarro et al., 2004; Eulgem, 2005; Burch-Smith et al., 2007; Dangl, 2007; Shen et al., 2007). Recognition of a MAMP by a cell surface receptor leads to activation of WRKY transcription factors through a MAP kinase cascade (Asai et al., 2002). Recent studies have revealed that many R-proteins function by directly modulating activities of transcription factors (Deslandes et al., 2002; Holt et al., 2002; Deslandes et al., 2003; Shen et al., 2007; Shen and Schulze-Lefert, 2007). These and other studies together indicate that different signaling events triggered by pathogen recognition converge in the cell nucleus to modify transcriptional factors that regulate both the basal and the R-mediated defense responses.

In addition to GPA1, the *Arabidopsis* genome encodes three extra-large GTP binding proteins (XLG1, XLG2, and XLG3) (Lee

and Assmann, 1999; Ding et al., 2008). To date, XLG genes have been found in plant genomes but not elsewhere (Ding et al., 2008). The C-termini of XLGs are similar to prototypical $G\alpha$ proteins. The N-termini (~400 amino acids) are homologous to each other but share little sequence similarity to other known proteins in the GenBank database. Recently, the *xlg2 xlg3* double mutant and the *xlg1 xlg2 xlg3* triple mutant were found to display an increased primary root length when grown in the dark (Ding et al., 2008), and insertional mutation of XLG3 impaired root waving and skewing (Pandey et al., 2008). The triple mutant also exhibited altered sensitivity in root growth and/or seed germination in response to ABA, osmotic stress, ethylene, and sugars. In addition, these XLGs were found to be localized in the nucleus by transient transformation (Ding et al., 2008).

We initially identified XLG2 as one of the early pathogen-responsive (EPR) genes from a transcriptome profiling analysis. In a subsequent phenotype screen for alteration in resistance to *P. syringae* in knockout mutants of over 50 EPR genes, the *xlg2* mutant was identified as an enhanced disease susceptibility mutant. Our study revealed that XLG2 physically interacts with AGB1 in planta. Constitutive overexpression of XLG2 was found to cause constitutive accumulation of abnormal transcripts from defense-related genes. The results indicate that XLG2 is a component of the G-protein heterotrimer and may be involved in transcriptional or post-transcriptional regulation of defense-related genes.

RESULTS

The *xlg2* Mutation Results in Enhanced Susceptibility to *Pseudomonas syringae*

We have obtained T-DNA insertion lines for over 50 *Arabidopsis* genes that were rapidly induced following infection with the *P. syringae* pv. *tomato* strain DC 3000 (*Pst*) (Ge et al., 2007). These lines were screened using an in-planta bacterial growth assay to search for any mutation that alters resistance to *Pst*. Mutations in at least three of these genes led to alteration in the disease resistance phenotype. Among them, the loss-of-function mutation in the *AtNUDT7* gene resulted in enhanced resistance to the infection (Ge et al., 2007). *xlg2* is one of the two enhanced disease susceptibility mutants that were found in the screen to be more susceptible to *Pst* than wild-type (wt) plants. The *xlg2* mutation results in a moderate but significant increase (approximately five-fold) in the growth rate of *Pst* in multiple independent in-planta growth assays, as exemplified in Figure 1A. Growth of both the avirulent strain *Pst avrRpm1* as well as *P. syringae* pv. *phaseolicola* (*Psp*), a non-host strain on *Arabidopsis*, were also moderately increased in the *xlg2* mutant plants compared to wt plants (Figure 1B and 1C). We did not observe any difference between the mutant and wt plants in appearance of hypersensitive cell death triggered by *Pst avrRpm1* or *Pst avrRpt2*.

Northern blotting analysis showed that the XLG2 gene is expressed at a low level in uninfected leaves of wt plants

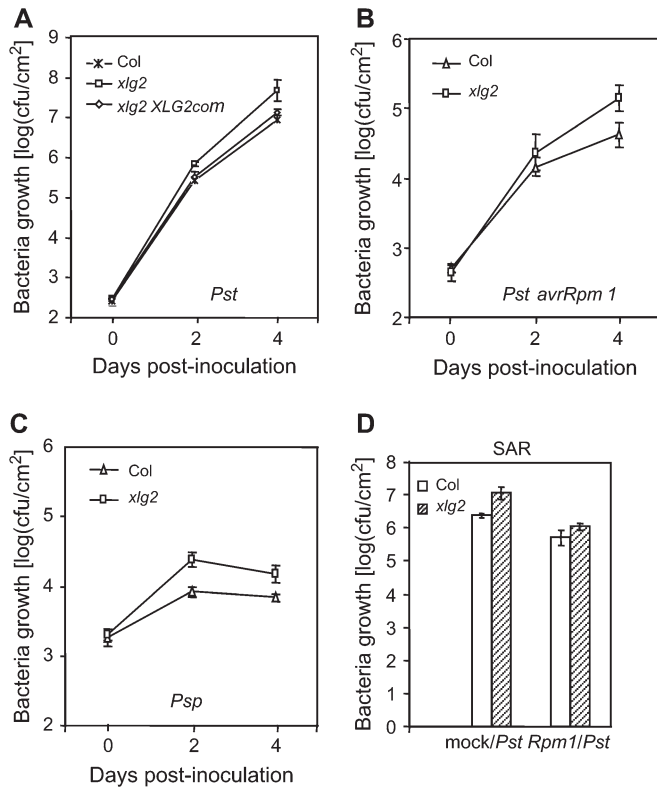


Figure 1. The *xlg2* Mutation Leads to Enhanced Disease Susceptibility.

(A) The in-planta growth assays show a six-fold increase in bacterial growth in the mutant over the wt plants (Col). Leaves were hand-infiltrated with the virulent *Pst* strain and bacterial numbers in the infected leaves were counted 2 and 4 d post inoculation. The mutant's enhanced disease susceptibility phenotype was complemented by the *XLG2com* transgene expressed in the *xlg2* mutant.

(B, C) The *xlg2* mutation also compromises resistance to the avirulent *Pst avrRpm1* strain (B) and to the non-host strain *Psp* (C).

(D) The *xlg2* mutant is not defective in induction of SAR. Induction of SAR by *Pst avrRpm1* led to an over five-fold decrease in growth of *Pst* in both the mutant and wt plants. *Rpm1/Pst*: four to five leaves of each plant were inoculated with *Pst avrRpm1* to induce SAR and, 3 d later, other upper leaves were infected with virulent *Pst*. Mock/*Pst*: no SAR induction prior to infection with *Pst*; plants had been mock-inoculated with water before infection with *Pst*. Shown are the bacterial colony forming units (cfus) in the leaves 4 d post infection with *Pst*. Each data point represents the average of three replicates \pm standard deviation.

(Figure 2A). Significant accumulation of the *XLG2* transcript was detected within 30 min of inoculation with *Pst avrRpm1* and reached its highest level 3 h post-inoculation (hpi). *XLG2* was also quickly induced by the virulent *Pst* strain; however, its transcript level dropped by 3 hpi. At later time points, the *Pst*-infected leaves produced a smaller transcript (indicated by the arrow in Figure 2A), which is estimated to be approximately 300 bp smaller than the normal *XLG2* transcript. It is unlikely that the smaller transcript was from cross-hybridization to *XLG1* or *XLG3* because it was not detected in the *xlg2* mutant

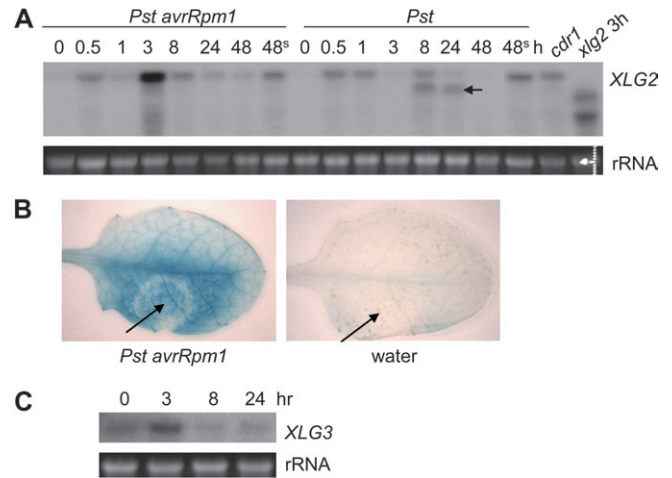


Figure 2. *XLG2* Is an Early Pathogen-Responsive Gene.

(A) Expression profiles of *XLG2* following infection with *Pst avrRpm1* and *Pst*. RNA was isolated from wt leaves (except the last two lanes) taken at different time points following pathogen inoculation. The two lanes labeled with 48^s are RNA samples of uninoculated leaves taken from the plants that had previously been inoculated with either *Pst avrRpm1* or *Pst*. *cdri*: the RNA sample was isolated from uninfected *cdri*-D mutant. *xlg2*, 3h: the RNA sample was isolated from the *xlg2* mutant leaves at 3 hpi with *Pst avrRpm1*. The arrow points to the smaller *XLG2* transcript.

(B) Induction of the *XLG2pro::GUS* reporter gene by *Pst avrRpm1* revealed by GUS staining. The leaves were taken from *XLG2pro::GUS* transgenic plants 20 hpi with the pathogen (left panel) or with H₂O as a negative control (right panel).

(C) *XLG3* is also pathogen inducible. RNA was isolated from leaves of wt plants taken at different time points after inoculation with *Pst avrRpm1*.

(Figure 2A). In addition, the hybridization was carried out under high stringency conditions meant to prevent such cross-hybridization. In the *cdri*-D mutant, an enhanced disease resistance mutant that expresses many defense-related genes in the absence of pathogen infection (Xia et al., 2004), the *XLG2* gene is constitutively expressed (Figure 2A).

As shown in Figure 2A, accumulation of the *XLG2* transcript was detected in the uninfected leaves of the plants inoculated with *Pst avrRpm1* or *Pst*, raising the possibility that *XLG2* could be involved in systemic acquired resistance (SAR). To determine whether the *xlg2* mutation affects induction of SAR, we first inoculated four to five leaves of each plant with *Pst avrRpm1* by hand-infiltration with bacterial suspension at a concentration of 2×10^7 cfu ml⁻¹ to induce SAR. Control plants were mock-inoculated (infiltrated with water). Three days later, un-inoculated leaves of these plants were infected with *Pst*. Growth of *Pst* was determined by counting bacterial numbers 4 d post-infection with *Pst*. In both wt and *xlg2* plants pre-inoculated with *Pst avrRpm1*, growth of *Pst* was reduced by more than five-fold compared to the mock-inoculated control plants (Figure 1D). This result indicates that the *xlg2* mutation does not impair induction of SAR. However, even with SAR induction, the growth rate of *Pst* in the *xlg2* mutant was still

higher than in wt plants, indicating that SAR induction did not compensate for the compromised local resistance caused by the *xlg2* mutation.

Transgenic lines expressing the GUS reporter under the control of the XLG2 promoter (XLG2pro) were generated. Staining for the presence of GUS activity in *Pst avrRpm1*-infected and mock-infected leaves of the XLG2pro::GUS lines further revealed that pathogen infection does induce expression of the reporter gene (Figure 2B, left panel). The mock-inoculated leaves (right panel) showed a detectable but weak GUS activity, whereas the pathogen-inoculated area (left panel, as indicated by the arrow) and the surrounding area exhibited a much stronger GUS activity.

The *xlg2* mutant line (SALK_062645) used in this study carries a T-DNA insertion in the second exon of the XLG2 gene (*At4g34390*). This T-DNA insertion line has previously been named as the *xlg2-1* allele (Ding et al., 2008), and we use *xlg2* to represent this mutant allele in this report. The *xlg2* mutant does not produce normal XLG2 transcripts in either uninfected leaves or leaves infected with *Pst avrRpm1* (Figure 2A), but rather produces two transcripts of smaller size when inoculated with *Pst avrRpm1* (Figure 2A). The exact nature and role (if any) of these transcripts remain to be determined; however, no protein bands were detected from these plants by Western analysis using the anti-XLG2 antibodies (see below). To confirm that the enhanced disease susceptibility phenotype associated with the *xlg2* mutant is indeed caused by disruption of XLG2 by the T-DNA insertion, we cloned the whole genomic fragment of this gene (including its 1.2-kb promoter region) from wt plants (Col-0 ecotype) and transformed it into the *xlg2* mutant. The transgene (XLG2com) was found to be able to complement the mutant phenotype as revealed in the in-planta bacterial growth assay (Figure 1A). Two independent XLG2com lines were tested in the assay and both lines showed a disease resistance phenotype indistinguishable from wt plants. Shown in Figure 1A is the result from one of the XLG2com lines.

XLG3 Is Also Pathogen-Inducible

Our previous GeneChip microarray data showed that the XLG1 transcript level was not significantly changed whereas the XLG3 transcript level increased 2.3-fold ($p < 0.01$) after infection with *Pst avrRpm1* (at 6 hpi) in wt plants. The pathogen-triggered induction of XLG3 was confirmed through RNA blot analysis (Figure 2C). However, two *xlg3* T-DNA insertion lines (SALK_030162/*xlg3-2* and SALK_141914/*xlg3-3*) and the *xlg1-1* mutant (Ding et al., 2008) showed no difference in resistance to *Pst* from wt plants. Although the in-planta growth rates of *Pst* in the *xlg2 xlg3-1* double mutant (Ding et al., 2008) were slightly higher than those in the *xlg2* single mutant, the differences were often not statistically significant. Similarly, the *xlg1-1 xlg2 xlg3-1* triple mutant (Ding et al., 2008) was found to be no different from either the *xlg2* single mutant or the *xlg2 xlg3-1* double mutant in the disease resistance phenotype. Furthermore, we did not find any significant difference between wt plants, the *xlg1-1*, *xlg2*, *xlg3-2*, *xlg3-3* single mutants,

the *xlg2 xlg3-1* double mutant, and the triple mutant in resistance to two necrotrophic fungal pathogens, *Botrytis cinerea* and *Alternaria brassicicola*.

The *xlg2* Mutation Compromises Induction of a Small Set of Pathogen-Responsive Genes

We initially used Northern blot analysis to determine whether the *xlg2* mutation affects induction of pathogen-responsive genes. We compared expression profiles of several well known defense-related genes between the mutant and wt plants following inoculation with *Pst avrRpm1* and/or *Pst*. These genes include PATHOGENESIS-RELATED GENE 1 (PR1), PR2, PR4, AvrRpt2-INDUCED GENE 1 (AIG1), and MITOGEN-ACTIVATED PROTEIN KINASE 3 (MPK3). Induction of PR1 and PR2 genes was found to be slightly weaker in the mutant following the *Pst avrRpm1* infection (Figure 3A) but expression patterns of the other genes were not different between wt and the *xlg2* mutant (data not shown).

We then carried out a gene expression profiling experiment using Affymetrix's *Arabidopsis* gene expression chip ATH1. RNA used to hybridize the GeneChip was isolated from leaves of wt and *xlg2* mutant plants 6 hpi with *Pst avrRpm1*. Six Affymetrix ATH1 arrays were used to hybridize the RNA samples, which included three biological replicates each for wt and

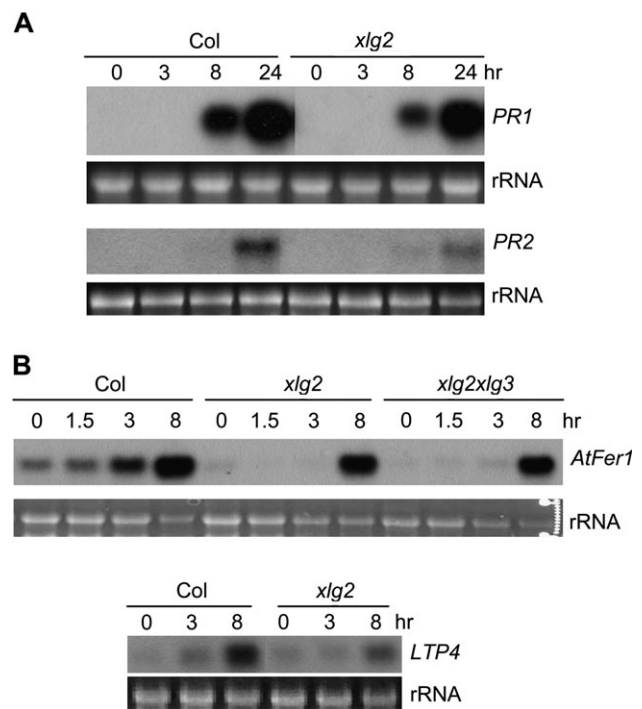


Figure 3. The *xlg2* Mutation Affects Pathogen-Triggered Expression of Defense-Related Genes.

RNA samples were isolated from leaves infected with *Pst avrRpm1* and uninfected leaves of wt and *xlg2* mutant plants at the indicated times post infection. RNA blotting analysis was used for the detection of the transcripts. *AtFer1*: At5g01600; *LTP4*: At5g59310.

the *xlg2* mutant. Analyses of the transcriptome data revealed that the overall gene expression profiles in the *xlg2* mutant and wt plants following pathogen infection were highly similar. Transcript levels of only a small set of genes were affected by the *xlg2* mutation. From the subset of differentially expressed genes that were identified with *p*-values of less than 0.05, those whose expression levels differed by more than two-fold are listed in Table 1. Among them, 17 genes (including *XLG2* and *PR1*) were expressed in the mutant at levels lower than in the wt plants, whereas another six genes were expressed at higher levels in the mutant. Expression patterns of some of the differentially expressed genes (including *AtFer1* and *LTP4*) were further confirmed by RNA blotting analysis (Figure 3B). Eleven of the 17 genes whose expression was impaired in the *xlg2* mutant were previously found to be pathogen-inducible according to the result from a separate Genechip transcriptome experiment (Table 1; see Methods).

XLG2 Protein Turnover Is Regulated via the Proteasome-Mediated Protein Degradation Pathway

Polyclonal antibodies against a 14-amino acid peptide derived from XLG2 were raised, affinity-purified, and used to detect XLG2 in protein extracts through immunoblot analysis. The

XLG2 level in crude protein extracts from uninfected leaves was below the limit of detection. In crude protein extracts from *Pst avrRpm1*-inoculated leaves, XLG2 protein was detectable, although faintly, by Western analysis (Figure 4A). No XLG2 protein was detected in the *xlg2* mutant, as expected. We have generated *XLG2* overexpression lines in which the *XLG2* gene is under the control of the 35S promoter (35S). Figure 4B shows a high *XLG2* transcript level in one of the 35S::*XLG2* lines (35S::*XLG2*-1), as determined by RNA blot analysis (Figure 4B). The XLG2 protein level was also significantly higher in the overexpression line than in wt plants infected with the avirulent pathogen (Figure 4A). However, even protein extracts from the overexpression line required a long (~5 min) exposure to X-ray film after incubation of the peroxidase-conjugated secondary antibody with its substrate in order to view the XLG2 protein band. Such a long exposure time led to the appearance of other protein bands that cross-reacted with the antibodies (Figure 4A).

In addition to the 35S::*XLG2* lines, we generated transgenic *Arabidopsis* lines that carry the 35S::*XLG2*-GFP fusion construct. However, the GFP signal was very weak in these lines when observed by confocal microscopy, suggesting that these plants do not accumulate a sufficient level of the fusion protein.

Table 1. A List of Genes that Were Differentially Expressed between *Pst avrRpm1*-Infected wt and *xlg2* Mutant Leaves.

Probe ID	Gene ID	Description of gene products	Fold difference	
			wt/ <i>xlg2</i>	Inf/uninf ¹
253257_at	At4g34390	XLG2	0.03	8.80*
251109_at	At5g01600	FERRITIN 1 (AtFer1)	0.15	1.89*
247718_at	At5g59310	non-specific lipid transfer protein (LPT4)	0.18	1.56
253044_at	At4g37290	Unknown	0.29	2.19*
247717_at	At5g59320	non-specific lipid transfer protein (LPT3)	0.38	0.59*
264514_at	At1g09500	cinnamyl alcohol dehydrogenase-like	0.38	12.33*
254098_at	At4g25100	Fe superoxide dismutase	0.38	0.74
267168_at	At2g37770	similar to aldo/keto reductase	0.41	10.11*
252984_at	At4g37990	cinnamyl alcohol dehydrogenase /ELI3-2	0.41	104.10*
255127_at	At4g08300	Nodulin MtN21 family protein	0.43	1.89*
266385_at	At2g14610	PR1	0.44	76.32*
263228_at	At1g30700	Reticuline oxidase-like	0.47	19.73*
253103_at	At4g36110	Auxin-induced protein	0.47	2.67*
265012_at	At1g24470	similar to b-keto acyl reductase	0.48	0.44*
251438_s_at	At3g59930	Defensin family protein	0.48	1.35
247492_at	At5g61890	unknown	0.48	1.50*
248205_at	At5g54300	unknown	0.50	1.10
246573_at	At1g31680	copper amine oxidase-like	2.39	0.59*
266590_at	At2g46240	BAG homolog	2.39	1.88*
246603_at	At1g31690	copper amine oxidase-like	2.47	0.55*
259511_at	At1g12520	copper/zinc superoxide dismutase	2.63	1.23
265058_s_at	At1g52040	myrosinase-binding protein	2.71	2.62*
266165_at	At2g28190	copper/zinc superoxide dismutase	3.02	0.73

¹ The numbers in this column represent the fold difference in transcript levels of these genes between *Pst avrRpm1*-inoculated and mock-inoculated leaves of wt plants at 6 hpi. The * indicates that the difference is statistically significant (*p* < 0.01).

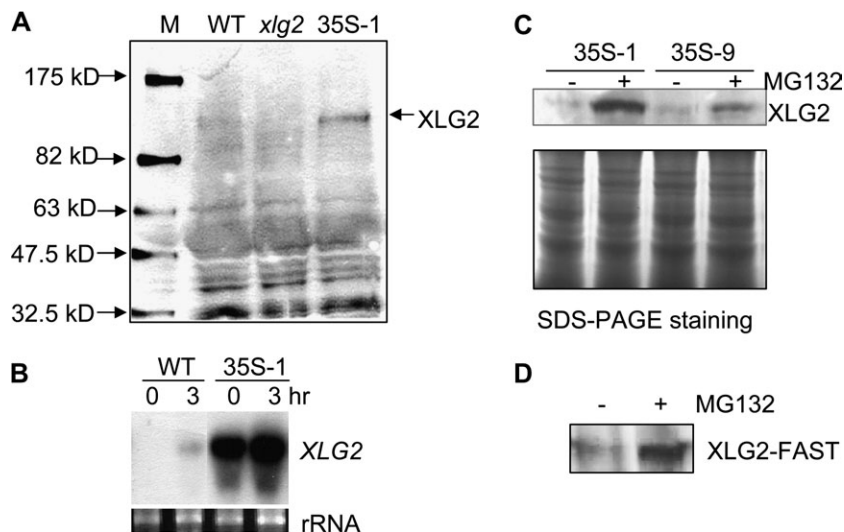


Figure 4. The XLG2 protein level is regulated by the proteasome-mediated protein degradation pathway.

(A) Detection of XLG2 protein with anti-XLG2 polyclonal antibodies through immunoblot analysis. Crude protein extracts were from leaves of wt, *xlg2*, and 35S::XLG2 transgenic line 35S-XLG2-1 (indicated as 35S-1 in the panel) plants 3 hpi with *Pst avrRpm1*. M, protein molecular weight markers.

(B) RNA blot showing XLG2 transcript levels in wt and the XLG2 overexpression line. Total RNA was isolated from uninfected (0 h) and infected (3 hpi with *Pst avrRpm1*) leaves of wt and 35S::XLG2-1 plants.

(C) MG132 treatment increased the XLG2 level in the two 35S::XLG2 lines (35S::XLG2-1 and 35S::XLG2-9 indicated as 35S-1 and 35S-9, respectively). MG132 (+) and water (–, as a negative control) were hand-infiltrated into leaves followed by protein extraction from the leaves 4 h after the infiltration. XLG2 protein was detected by immunoblot analysis (upper panel) using the anti-XLG2 polyclonal antibodies. The lower panel shows a Coomassie blue-stained gel with these protein samples.

(D) MG132 treatment increased the level of the XLG2-FAST fusion protein in the pathogen-infected leaves of XLG2pro::XLG2-FAST transgenic plants. Leaves were infiltrated with *Pst avrRpm1* alone (–) or the pathogen together with MG132 (+). Protein was extracted 4 hpi and detected using the anti-FLAG M2 antibody.

Together, these results suggest that the XLG2 protein level may be under tight control through translational and/or post-translational mechanisms. To determine whether XLG2 protein accumulation is subjected to regulation via the proteasome-mediated protein-degradation pathway, we infiltrated leaves with the synthetic proteasome inhibitor benzyloxycarbonyl-L-leucyl-L-leucyl-L-norvaline 4-methyl-coumaryl-7-amide (MG132) to block the proteasome activity. It was found that MG132 treatment significantly enhanced XLG2 accumulation in the XLG2 overexpression lines (Figure 4C). Similarly, in the XLG2pro::XLG2-FAST lines in which XLG2 is fused with the FAST epitope tag that consists of the FLAG tag and StrepII tag (Ge et al., 2005), the MG132 treatment also led to a much higher level of XLG2-FAST protein (Figure 4D).

XLG2 Interacts with AGB1 In Planta

A hallmark of canonical G α proteins is their interaction with G $\beta\gamma$ subunits, and, like the XLGs (Ding et al., 2008), the *Arabidopsis* G β subunit (AGB1) has been reported to be present in the nucleus (Obrdlik et al., 2000; Peskan and Oelmüller, 2000; Anderson and Botella, 2007). To determine whether XLG2 interacts with the *Arabidopsis* G β subunit of the heterotrimeric G-protein, we first used the yeast two-hybrid system. However, we were unable to detect any interaction between XLG2 and AGB1 in the yeast system. We then carried out a co-

immunoprecipitation (co-IP) assay to determine whether XLG2 interacts with AGB1 in planta. We fused the epitope tag FAST to the C-terminus of AGB1 and generated *Arabidopsis* transgenic lines expressing the AGB1-FAST fusion protein under the control of the 35S promoter. Expression of AGB1-FAST in the transgenic lines was confirmed through immunoblot analysis of crude protein extracts from the transgenic plants using the anti-FLAG M2 monoclonal antibody (Figure 5A). For the co-IP experiment, the transgenic plants were infected with *Pst avrRpm1* to induce expression of XLG2. AGB1-FAST was then immuno-precipitated from crude protein extracts of the infected leaves by its affinity to the anti-FLAG M2 antibody. AGB1-FAST, which has a predicted molecular mass of 41 kDa, was not visible from the immuno-precipitate in the Coomassie-stained SDS-PAGE gel; however, it was readily detected in the immuno-precipitate through immunoblot analysis (Figure 5A). We then used the anti-XLG2 polyclonal antibodies to detect XLG2 in the AGB1-containing immuno-precipitate. As shown in Figure 5B, XLG2 was also readily detected in the precipitate, demonstrating that XLG2 was co-precipitated with AGB1-FAST. We tried to determine if there is any interaction between AGB1 and XLG2 in uninfected leaf tissues using the same method; however, we could not detect any XLG2 in the precipitate, presumably because of an insufficient level of XLG2 in uninfected tissues, although we could not

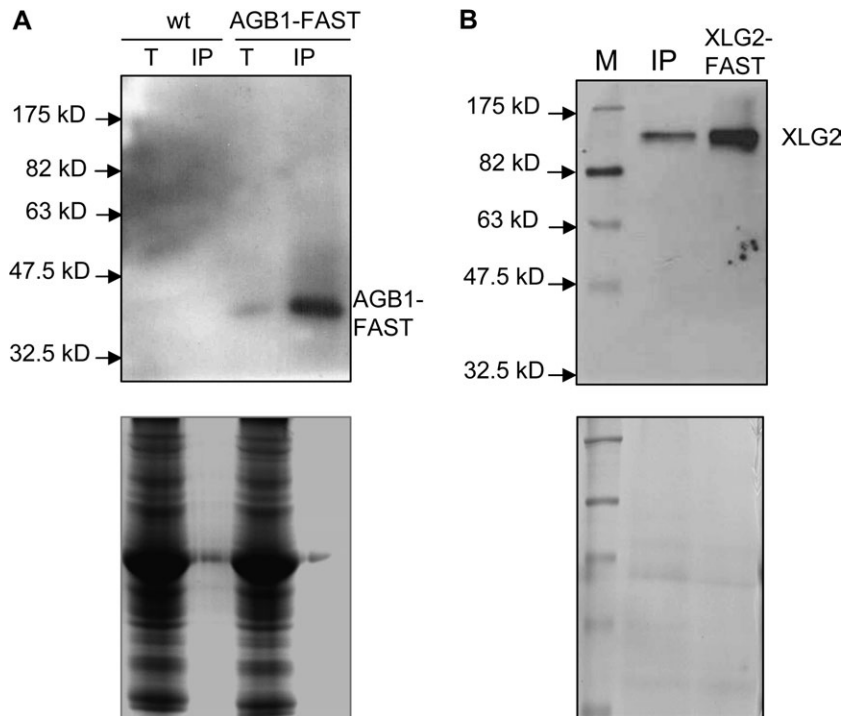


Figure 5. Co-Immunoprecipitation Assay Reveals that XLG2 Interacts with AGB1 In Planta. Proteins were extracted from leaves of the 35S::AGB1-FAST transgenic line (AGB1-FAST) and non-transgenic wt plants (wt) infected with *Pst avrRpm1*. T, total protein extract (wt); IP, immunoprecipitated protein.

(A) Detection of AGB1-FAST in the total protein extracts and the immunoprecipitates with the anti-FLAG M2 antibody in immunoblot analysis (upper panel). The lower panel shows Coomassie blue staining of the SDS-PAGE gel containing the same protein samples.

(B) Detection of XLG2 in the proteins co-immunoprecipitated by anti-FLAG M2 monoclonal antibody against AGB1-FAST. XLG2 presence in the immunoprecipitate (IP) was detected with anti-XLG2 polyclonal antibodies (upper panel). XLG2 protein in the precipitate was readily detectable. The lane labeled 'XLG2-FAST' contains XLG2-FAST protein, which was pulled down from 35S::XLG2-FAST transgenic plants using the anti-FLAG M2 antibody affinity gel, as a positive control for XLG2 detection in the immunoblot analysis. The lower panel shows Coomassie blue staining of the SDS-PAGE gel containing the same protein samples as the immunoblot.

rule out the possibility that XLG2 may not interact with AGB1 in uninfected tissues.

Constitutive Overexpression of XLG2 Leads to Constitutive Accumulation of Abnormal Transcripts from Defense-Related Genes

Two *XLG2* overexpression lines (35S::*XLG2*-1 and 35S::*XLG2*-9) were subjected to disease resistance phenotype analysis. None of the *XLG2* overexpression lines showed any visible morphological difference from a wt plant. Nor did we find any difference between the overexpression lines and the wt plants in resistance to *Pst* or to the fungal pathogens *B. cinerea* and *A. brassicicola*. We also compared expression patterns of six well known pathogen-responsive genes between the overexpression lines and wt plants through RNA blot analysis. We did not find any significant difference in expression patterns of three of the genes (*PR1*, *PR4*, and *AIG1*) that were probed in the analysis (Figure 6). However, both *XLG2* overexpression lines showed constitutive accumulation of transcripts from three other defense-related genes (*AtMPK3/At3g45640*, *RbohC/At5g51060*, and *PAD3/At3g28630*; Figure 6). Surprisingly, all three of these genes generated abnormally small transcripts in the overexpression lines. In the case of the *RbohC* gene, the smaller transcript (estimated to be 0.8–1 kb smaller than the normal transcript) appeared in both pathogen-infected wt tissues and the overexpression line, whereas the smaller transcripts from *MPK3* (estimated to be 300–500 bp smaller than the normal transcript) and *PAD3* were only detected in the overexpression lines. To investigate whether

these abnormal transcripts result from alternative splicing, we attempted to amplify the alternative transcripts of these genes through reverse transcription PCR (RT-PCR) from RNA samples isolated from the *XLG2* overexpression lines. Although multiple sets of primer combinations were used in the PCR analysis (Supplementary Data), we only detected RT-PCR products that have predicted normal sizes. The failure to detect alternatively spliced transcripts suggests that these abnormal transcripts may be derived from alternative transcription initiation or termination. It is also possible that these abnormal transcripts may be derived from an RNA processing mechanism that specifically removes either a 5' or 3' portion of the transcripts.

DISCUSSION

Basal resistance, analogous to horizontal resistance, is largely a quantitative trait controlled by multiple genes (Young, 1996). Several important genes involved in basal as well as R-mediated resistance, including *NPR1*, *EDS1*, *PAD4*, and *RAR1*, have been identified through genetic screens (Cao et al., 1997; Falk et al., 1999; Jirage et al., 1999; Shirasu et al., 1999). That being said, mutations in many of as yet unknown genes that regulate basal immunity may result in only a minor change in the disease resistance phenotype, making it difficult to identify them through a classical forward genetic approach. The loss-of-function mutation of *XLG2*, which encodes one of the three unique Gα-like XLG proteins, was found to cause moderately enhanced susceptibility to *P. syringae* in a reverse genetic screen aiming to identify

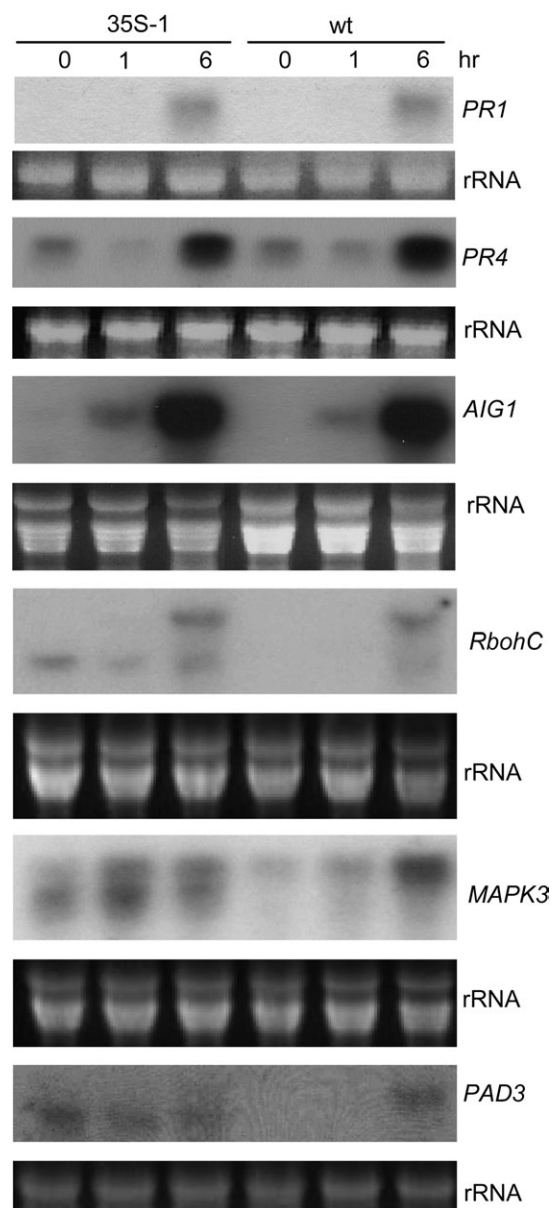


Figure 6. Constitutive Overexpression of XLG2 Leads to Constitutive Accumulation of Abnormal Transcripts of a Few Defense-Related Genes.

Transcript profiles of *PR1*, *PR4*, and *AIG1* are similar between wt and the overexpression line 35-XLG2-1 (indicated as 35S-1); however, abnormal transcripts from three other genes were detected from the XLG2 overexpression line. RNA was isolated from uninfected leaves (0 h) and leaves infected with *Pst avrRpm1*, and evaluated with probes for defense-related genes in RNA blot analysis. The detection of *PAD3* was carried out using Roche's DIG High Prime DNA labeling and Detection kit and the probes for all other genes were labeled with ^{32}P .

genes that function in basal immunity. The *xlg2* mutation also compromises resistance to an avirulent strain of *Pst* and to the non-host strain *Psp*. Together, the results indicate that XLG2 has a positive regulatory role in the plant defense response

against the bacterial pathogen. In addition to XLG2, transcription of *XLG3* is induced by pathogen infection. However, the *xlg2 xlg3* double mutant and the *xlg1 xlg2 xlg3* triple mutant were not found to be significantly different from the *xlg2* mutant in the tested disease resistance phenotypes.

Heterotrimeric G-proteins are involved in transducing extracellular signals from cell surface receptors into intracellular effectors (Neer, 1995; Neves et al., 2002). Although plants have only a small number of genes encoding the G-protein subunits, they have been implicated in a wide variety of biological processes (for reviews, see Jones and Assmann, 2004; Perfus-Barbeoch et al., 2004). Until now, very few downstream effectors of G-proteins have been identified from plants, and the molecular mechanisms by which G-proteins activate downstream signaling pathways in plant biological processes remain elusive. It has been proposed that a G-protein may activate a signaling event in response to stress conditions that lead to activation of NADPH oxidase(s) and production of reactive oxygen species (Suharsono et al., 2002; Joo et al., 2005).

All three regular subunits of the *Arabidopsis* G-protein have been reported to have roles in disease resistance. The *Arabidopsis gpa1* mutant exhibited enhanced resistance to several necrotrophic fungal pathogens, including *Plectosphaerella cucumerina*, *A. brassicicola*, and *Fusarium oxysporum* (Llorente et al., 2005; Trusov et al., 2006). In contrast, mutations in *AGB1* and *AGG1* lead to enhanced susceptibility to necrotrophic fungal pathogens (Llorente et al., 2005; Trusov et al., 2006, 2007). These results suggest that the $G\alpha$ subunit is a negative regulator while $G\beta$ and $G\gamma$ are positive regulators in disease resistance. However, the rice $G\alpha$ loss-of-function mutation (the *d1* mutation) compromises resistance against an avirulent strain of the biotrophic fungal pathogen *Magnaphorthe grisea*, which causes rice blast (Suharsono et al., 2002). The *d1* mutant is impaired in the hypersensitive response, H_2O_2 production, and defense gene induction triggered by the pathogen (Suharsono et al., 2002). In *Arabidopsis*, neither the $G\alpha$ mutation nor the $G\beta$ mutation alters resistance to virulent or avirulent strains of *P. syringae*, a biotrophic bacterial pathogen (Trusov et al., 2006). These studies suggest that the role of G-protein in plant disease resistance depends on the type of pathogen (necrotrophic versus biotrophic and bacterial versus fungal) and that the different G-protein subunits may have different functions. The molecular mechanism by which the various subunits of G-proteins affect disease resistance also remains obscure. The *agb1* mutant was found to be impaired in induction of *PDF1.2* by either methyl jasmonate (MeJA) or *A. brassicicola* (Trusov et al., 2006). Our study revealed that XLG2 interacts with AGB1 and acts a positive regulator in resistance to *P. syringae* that largely triggers the SA response pathway. However, we did not observe any obvious differences between wt plants and any of the *xlg* mutants (including their double and triple mutants) in resistance to *B. cinerea* or *A. brassicicola*, which trigger the JA-responsive pathway, suggesting that XLG2 and AGB1 have distinct, and possibly antagonistic, functions in disease resistance through their interaction.

Because of significant sequence divergence between the XLGs and the canonical $G\alpha$, it was proposed by Temple and Jones (2007) that the XLGs are not likely to interact with the $G\beta\gamma$ subunit in a traditional fashion, if at all. In the yeast two-hybrid system, we could not detect interaction of XLG2 with AGB1; however, the co-immunoprecipitation assay demonstrated that XLG2 does interact with AGB1 in planta. These results indicate that interaction of XLG2 with AGB1 may be regulated through certain conformational changes that could be mediated through an intramolecular event (such as protein modification) or an intermolecular event (such as interaction with another protein).

The N-termini of the three XLGs contain a putative nuclear localization signal, and GFP fused with XLGs is localized in the nuclei in *Vicia faba* leaves as determined by a transient expression assay (Ding et al., 2008). The heterotrimeric forms of canonical G-proteins are generally associated with the plasma membrane, and localization of GPA1, AGB1, AGG1, and AGG2 at the plasma membrane has been confirmed (Adjobo-Hermans et al., 2006; Zeng et al., 2007; Wang et al., 2008). Accordingly, the discovery that XLGs localize in the nucleus suggests that XLGs might have a different mode of action from canonical $G\alpha$. However, the subunits of the canonical G-protein in plants have also been found in multiple compartments. For instance, the $G\beta$ subunit has also been reported to be targeted to the nucleus when expressed in both *Arabidopsis* and tobacco cells (Obrdlik et al., 2000; Peskan and Oelmüller, 2000; Anderson and Botella, 2007). In another report, AGB1 was found to be largely associated with the endoplasmic reticulum (ER) and to play a role in the unfolded protein response (Wang et al., 2007). It is tempting to speculate that XLG2 may mediate information transfer from cell surface to nucleus in response to an extracellular signal such as pathogen recognition. In animals, $G\beta 5$ undergoes nucleocytoplasmic shuttling, which is mediated by binding of regulators of G-protein signaling (RGS) to $G\beta 5$ (Rojkova et al., 2003).

The gene expression microarray data show that overall defense transcriptomes in pathogen-infected leaves are very similar between the *xlg2* mutant and wt plants. About 24 genes showed more than a two-fold difference in their expression levels between the mutant and wt plants. Most of these differentially expressed genes are pathogen-inducible. Although the result suggests that the *xlg2* mutation alters defense gene induction, this small set of differentially expressed genes does not offer a clear insight into which particular defense signaling pathway is affected by the *xlg2* mutation. Interestingly, constitutive overexpression of XLG2 leads to constitutive expression of three defense-related genes that were examined by the Northern blot analysis (*MPK3*, *RbohC*, and *PAD3*). Surprisingly, the accumulated transcripts of these three genes had abnormal sizes in the overexpression lines. These abnormal transcripts may be derived from alternative transcription initiation or termination or could be a product of RNA processing. The irregularity in transcriptional and/or post-transcriptional processing of these genes through the overexpression of

XLG2, together with XLG2 localization in the nucleus, raise the possibility that XLG2 is a component of transcriptional and/or post-transcriptional regulation of defense-related genes.

METHODS

Plant Materials and Growth Conditions

The wild-type *Arabidopsis* plants used in this study are the Columbia (Col-0) ecotype. The *xlg2-1* (SALK_062645) and *xlg3-2* and *xlg3-3* T-DNA insertion mutants (SALK_030162 and SALK_141914) were obtained from the Arabidopsis Biological Resource Center (Columbus, OH). The *xlg1-1* single mutant, *xlg2-1 xlg3-1* double mutant, and *xlg1-1 xlg2-1 xlg3-1* triple mutant were previously described (Ding et al., 2008). *Arabidopsis* plants used in the experiments were grown in a growth room with the following conditions: an 8-h light/16-h dark light cycle at a light intensity of $125 \text{ mol m}^{-2} \text{ s}^{-1}$ provided by cool-white fluorescent bulbs, 50% humidity and 21°C .

PCR Verification of T-DNA Insertions at XLG2 and XLG3

The T-DNA insertion in SALK_62645 was verified by PCR analysis using a T-DNA left-border primer (LBb1, 5'-GCGTGGACCGC-TTGCTGCAACT-3') and a XLG2-specific primer (XLGp1, 5'-AACTGGCAGAGAGAACACAGC-3'). The primers LBb1 and XLG3p1r (5'-TGGCTGCAAATGAAGCCTAA-3') were used to amplify the insertion-flanking sequence in SALK_030162 while the primer pair LBb1 and XLG3p3r (5'-GGGGTGCTTAACCATGATTGCG-3') were used to amplify the insertion flanking sequence in SALK_141914.

RNA Blot Analysis

Total RNA was isolated from leaf tissues using the Trizol extraction buffer (Invitrogen, Carlsbad, CA) and the subsequent electrophoresis, probe labeling, and Northern blotting analysis was conducted according to standard procedures (Sambrook et al., 1989). A new RNA blot was used for each probe. DNA fragments used for making probes in RNA blot analysis were amplified from the genomic DNA using the following primer pairs: 5'-ATGAACACCGGCGGTGGCCAATAC-3' and 5'-GCTCG-GCTTTAAATCCCTATGAATAATG-3' for *AtMPK3*, 5'-AATGA-AAGTTTCAGTATCAC-3' and 5'-GCAGTTAACTTGTGTGGC-3' for *WAK1*, 5'-ATGTCTAGAGTGAGTTTTGAAG-3' and 5'-TCC-GGTCTAACTTAGCCGGTC-3' for *RbohC*, 5'-TTAGTCAACGTTA TGCGATGGGTC-3' and 5'-TGAAGAACTTGAAAGAAGGCTAG-AA-3' for *PAD3*, 5'-GGAGAGAGCGAGTAGGAAATAAG-3' and 5'-CATAGGAGACGTTGTATTCCACAT-3' for *AtFer1/At5g01600*, 5'-CCCAAAGAGAAGAGCAAACAC-3' and 5'-CTTACGTGGCG-CAGTTGGTG-3' for *LTP4/At5g59310*. The detection of *PAD3* was carried out using Roche's DIG High Prime DNA labeling and Detection kit (Indianapolis, IN) and the probes for all other genes were labeled with ^{32}P .

Microarray Gene Profiling and Data Analyses

Leaves of wt and the *xlg2* mutant plants were inoculated with *Pst avrRpm1* and collected for RNA isolation 6 h post-inoculation. RNA was isolated using the Trizol extraction method followed by RNA purification using the RNeasy Mini-Elute Cleanup kit (QIAGEN, Valencia, CA). Six Affymetrix ATH1 arrays were used to hybridize the RNA samples, including three biological replicates each for both wt and the *xlg2* mutant. cRNA synthesis, labeling, hybridization and scanning were carried out at Iowa State University GeneChip Facility. The original microarray data (.cel files) were background corrected, quantile normalized, and summarized for each probe set using the *affy* package (Irizarry et al., 2003) in BioConductor with default settings. The summary scores were then analyzed probe set by probe set using the MAANOVA package (Wu et al., 2003) in BioConductor. A shrinkage-based *t*-test was conducted to compare the two groups (Cui et al., 2005). An empirical *p*-value was obtained for each probe set based on permutation of the observed data (Yang and Churchill, 2007). Differentially expressed genes were identified with the constraint of *p*-values of less than 0.05. From this subset of genes, the genes that showed a fold-change of greater than two-fold are listed in Table 1. The fold changes of these genes between pathogen-inoculated and mock-inoculated plants shown in Table 1 are from the result of a separate Genechip experiment aimed to identify pathogen-responsive genes. In that experiment, leaves of wild-type plants (Col) were inoculated with *Pst avrRpm1* or water (control) and collected at 6 hpi for RNA extraction. Three biological replicates were included in the analysis. The methods for chip hybridization and data analysis are the same as above except that the false-discovery rate (FDR) was used as the multiple comparison correction. The genes that showed a significant difference in their transcript levels between the pathogen-infected sample and the control sample (with FDR-corrected *p*-values of less than 0.01) are indicated with * in Table 1.

GUS Activity Staining

GUS activity staining of leaves from the *XLG2pro::GUS* transgenic plants was carried out as previously described (Xia et al., 1997). Leaves were stained overnight (approximately 15 h), cleared in 100% ethanol overnight, and kept in 70% ethanol before images were taken.

Pathogen Treatment and MG132 Treatment

P. syringae pv. *tomato* (*Pst*), *Pst avrRpm1*, *Pst avrRpt2*, *P. syringae* pv. *phaseolicola* (*Psp*), and *P. syringae* pv. *maculicola* (*Psm*) were grown as previously described (Cameron et al., 1994). For in-planta growth assays, bacterial suspensions at a concentration of 5×10^4 cfu ml⁻¹ (for *Pst*), 1×10^5 cfu ml⁻¹ (for *Pst avrRpm1*), or 2×10^5 cfu ml⁻¹ (*Psp*) were hand-infiltrated into *Arabidopsis* leaves. To prepare tissues used for studying pathogen-induced expression of defense-related genes or proteins, leaves were infiltrated with bacterial suspensions at

a concentration of 2×10^7 cfu ml⁻¹. Mock inoculations were infiltrated with water.

For MG132 treatment, a 50-μM solution of MG132 (Sigma-Aldrich, St Louis, MO) was hand-infiltrated into leaves. In the pathogen and MG132 co-treatment experiment, MG132 was added to pathogen suspensions to a concentration of 50 μM and the mixture was then immediately infiltrated into the leaves.

Construction of the XLG2 Complementation Vector (*XLG2com*), *XLG2pro::GUS*, *XLG2 pro::XLG2-FAST*, *35S::AGB1-FAST*, and *35S::XLG2*

The *XLG2* genomic fragment, including the 1.3-kb promoter region, was PCR-amplified from Col plants by using the primer pair *XLG2p5Bam* (5'-GGATTCGCATGAAGGAAGTGGC-3') and *XLG2p3Sal* (5'-GTCGACCGATAATTTATTGCTACTCCG-3'), subcloned into pCR-*BluntII*-TOPO vector, verified by sequencing, excised by cutting with *Bam*HI and *Sal*I, and ligated into the binary vector pBI101.3 to generate *XLG2comp*. To construct *XLG2pro::GUS*, a pair of primers, (*XLG2p5Sal*, 5'-GTCGACGAGCCAGCAGCATCTTC-3') and (*XLG2p3Bam*, 5'-GGATCCCAATC-AAGCACACATACAAAC-3'), was used to amplify the 1.3-kb promoter sequence. The PCR fragment was subcloned into pCR-*BluntII*-TOPO, excised by cutting with *Sal*I/*Bam*HI, and inserted upstream of the GUS reporter gene in the binary vector pBI101.3. For construction of *XLG2pro::XLG2-FAST*, a ~4.5-kb *XLG2* genomic fragment, including its ~1.2-kb promoter region, was PCR amplified with the primer pair (5'-TCCC-CCGGGAAGTGGCGCTGGAGTTC-3' and 5'-GCTCTAGAAGAGGACGAGCTGGCCTC-3'), cloned into pCR-*BluntII*-TOPO, verified by sequencing, released by cutting with *Sma*I and *Xba*I, and subcloned into the *Sma*I/*Xba*I-digested vector pAKK-BAR-FAST, a plant binary vector constructed by modifying the epitope-tagging vector previously described (Ge et al., 2005). The clone results in *XLG2* fused in frame with the FLAG and *Stre*II tags. To construct *35S::AGB1-FAST*, the *AGB1* cDNA clone, including the entire ORF, was amplified with primers (5'-ATGGTACCATGTCTGTCTCCGAGCTC-3' and 5'-CTTCTAGAAATCACTCTCCTGTGTCTCCTC-3') by RT-PCR and cloned into pCR-*BluntII*-TOPO. The *Kpn*I/*Xba*I fragment was digested out and subcloned into *35S::pAKK-BAR-FAST* (a binary vector based on pAKK-BAR-FAST with the 35S promoter upstream of the FAST tag). The resulting clone was named *35S::AGB1-FAST*. Construction of *35S::XLG2* was carried out by amplifying the *XLG2* genomic fragment (without its promoter region) with the primer pair *XLG2p5BamC* (5'-GGATCCTTGATTGGGT-AAGAAGATGG-3') and *XLG2p3Sal*. The PCR product was cloned into pCR-*BluntII*-TOPO and then inserted downstream of the 35S promoter in the binary vector pCHF3 to generate *35S::XLG2*.

Arabidopsis Transformation

Arabidopsis was transformed via *Agrobacterium tumefaciens*-mediated transformation as previously described (Clough and Bent, 1998).

Immunoblot Assays and Antibodies

To isolate protein extract, *Arabidopsis* tissues were homogenized in Cellytic™ P Plant Cell Lysis/Extraction Reagent (Sigma-Aldrich) with 1× protease inhibitor cocktail (Sigma-Aldrich). The extract was centrifuged at 12 000 *g* at 4°C for 15 min. The supernatant was collected and the protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA). Protein samples were boiled in SDS-loading buffer, separated on SDS-PAGE gels and transferred onto nitrocellulose membranes (Osmonics, Gloucester, MA). For production of anti-XLG2 polyclonal antibodies, a peptide of XLG2 (KRLDVPEEVKSPADC) was synthesized and used for raising polyclonal antibodies in rabbits (carried out by Sigma-Genosys). The antibodies were affinity-purified with the peptide by Sigma-Genosys and used for immunoblot analyses at a 1:1000 dilution.

Co-Immunoprecipitation Assay

Leaves from 35S::AGB1-FAST transgenic plants and from non-transgenic wt plants were hand-infiltrated with *Pst avrRpm1* and collected at different time points (20 min, 30 min, 1 h, 3 h, and 6 h post infection). A mixture of infected leaves was used for the total protein extraction. Protein extraction and co-immunoprecipitation with the anti-FLAG M2 antibody gel (Sigma-Aldrich) were carried out according to the procedure previously described (Feng et al., 2004). Briefly, total protein extract was clarified by centrifuging at 12 000 *g* at 4°C for 15 min. Samples of 5–10 ml of supernatant were incubated with 50–100 µl of anti-FLAG affinity gel (Sigma) at 4°C for 4–5 h with gently shaking. The samples with resin were transferred into Pierce spin columns (cat. # 69705, Pierce, Rockford, IL) and spun at 300 *g* briefly to collect resin. After washing with the extraction buffer four times, protein bound to the affinity gel was eluted with 3 FLAG peptide (Sigma-Aldrich). The eluted immunoprecipitate was kept at –80°C before use in immunoblot analysis.

SUPPLEMENTARY DATA

Supplementary Data are available at *Molecular Plant Online*.

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